VERSION WITH MARKINGS TO SHOW CHANGES MADE

[009] Some years ago, a further method of DNA sequencing was developed on the basis of optical (or autoradiographical) detection, namely sequencing by oligomer hybridization (cf. e.g. Drmanac et al., Genomics 4, (1989), pp. 114-128 or Bains et al., Theor. Biol. 135, (1988), pp. 303-307). In this method, a complete set of short oligonucleotides or nucleic acid oligomers (probe oligonucleotides), e.g. all 65,536 possible combinations of bases A, T, C, and G of an oligonucleotide octamer, are bound to a support material. The attachment occurs in an ordered grid comprising 65,536 test sites, each rather large amount of an oligonucleotide combination defining one test site, and the position of each individual test site (oligonucleotide combination) being known. On such a hybridization matrix, the oligomer chip, a DNA fragment whose sequence is to be determined (the target) is labeled with fluorescent dye (or ^{32}P) and hybridized under conditions that allow only one specific double-strand formation. In this way, the target DNA fragment binds only to those nucleic acid oligomers (in this example to the octamers) whose complementary sequence corresponds exactly to a portion (an octamer) of its own sequence. Thus, all of the nucleic acid oligomer sequences (octamer sequences) present in the fragment are determined by optical (or autoradiographical) detection of the binding position of the hybridized DNA fragment. Due to the overlapping of neighboring nucleic acid oligomer sequences, the sequential sequence of the DNA fragment can be determined using appropriate mathematical algorithms. One of the advantages of this method lies in the miniaturization of the sequencing and thus in the enormous amount of data that can be simultaneously captured in one operation. In addition, primer and gel-electrophoretic separation of the DNA fragments can be dispensed with. This principle is exemplified in Fig. 1 for a 13-base-long DNA fragment **(SEQ ID NOS.: 2-18)**.

[020] The following abbreviations and terms will be used in the context of the present invention:

DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PNA	Peptide nucleic acid (synthetic DNA or RNA in which the sugar-phosphate moiety is replaced by an amino acid. If the sugar-phosphate moiety is replaced by the -NH-$(CH_2)_2$-N($COCH_2$-base)-CH_2CO- moiety, PNA will hybridize with DANN.)
A	Adenine
G	Guanine
C	Cytosine
T	Thymine
U	uracil
base	A, G, T, C, or U
bp	base pair
nucleic acid	At least two covalently-joined nucleotides or at least two covalently-joined pyrimidine (e.g. cytosine, thymine, or uracil) or purine bases (e.g. adenine or guanine). The term nucleic acid refers to any backbone of the covalently-linked pyrimidine or purine bases, such as the sugar-phosphate backbone of DNA, cDNA, or RNA, a peptide backbone of PNA, or analogous structures (e.g. a phosphoramide, thiophosphate, or dithiophosphate backbone). The essential feature of a nucleic acid according to the present invention is that it can sequence-specifically bind naturally occurring cDNA or RNA.
nucleic acid oligomer	Nucleic acid of a base length that is not further specified (e.g. nucleic acid octamer: a nucleic acid having any backbone in which 8 pyrimidine or purine bases are covalently bound to one another).
oligomer	Equivalent to nucleic acid oligomer.
oligonucleotide	Equivalent to oligomer or nucleic acid oligomer, e.g. a DNA, PNA, or RNA fragment of a base length that is not further specified.
oligo	Abbreviation for oligonucleotide.

primer	Initial complementary fragment of an oligonucleotide, the base length of the primer being only approx. 4-8 bases. Serves as the starting point for enzymatic replication of the oligonucleotide.
mismatch	To form the Watson-Crick double-stranded oligonucleotide structure, the two single-strands hybridize in such a way that the A (or C) base of one strand forms hydrogen bonds with the T (or G) base of the other strand (in RNA, T is replaced by uracil). Any other base pairing does not form hydrogen bonds, distorts the structure and is referred to as a "mismatch".
ss	single-strand
ds	double-strand
redox-active moiety	Equivalent to a catalytically redox-active moiety.
catalytically redox-active moiety	In the context of the present invention, a moiety referred to using the generic term "catalytically redox-active moiety" usually consists of one or more redox-active centers (cofactors, prosthetic groups), which are referred to in the following as electron donors or electron acceptors, and one or more macromolecules binding these redox-active centers. Thus, in its form that is relevant to the present invention, the catalytically redox-active moiety includes one or more electron-donor molecules and/or one or more electron-acceptor molecules, this (these) electron-donor molecule(s) and/or this (these) electron-acceptor molecule(s) being/becoming bound to one or more macromolecules or being embedded in this (these) macromolecule(s). Electron donor(s) and/or electron-acceptor(s) may be joined with one another via one or more covalent or ionic bonds, via hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance, covalent links being able to be direct or indirect (e.g. via a spacer, but not via a nucleic acid oligomer) links. In addition, the electron donor(s) and/or electron acceptor(s) may be joined with the macromolecule(s) via covalent attachment to the macromolecule(s), via encapsulation in suitable molecular cavities (binding pockets) of the macromolecule(s), via ionic bonds, hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of

electron-pair donation and acceptance between the macromolecule(s) and the electron-donor molecule(s) and/or electron-acceptor molecule(s). If the catalytically redox-active moiety is composed of multiple macromolecules, the binding of the macromolecules to one another may likewise take place covalently, ionically, via hydrogen bonds, van der Waals bridges, π-π-interaction, or via coordination by means of electron-pair donation and acceptance. In the minimum case, a catalytically redox-active moiety may also consist of only one macromolecule, the macromolecule, in its form that is relevant to the present invention, then also acting as the electron donor or acceptor. It may also consist of only one electron donor or acceptor. In addition, the catalytically redox-active moiety may also be formed through spontaneous congregation of the components in solution (in situ). In addition to comprising electron donor(s) and/or electron acceptor(s) and macromolecule(s), essential features of the catalytically redox-active moiety are: (i) in the forms relevant to the present invention (electron donor(s) and/or electron acceptor(s) in their original state or in an oxidized or reduced state), the moiety is stable and does not dissociate into its components, (ii) the electrocatalytic activity of the moiety (see below), (iii) the moiety includes no nucleic acid, (iv) the moiety's composition comprising electron donor(s) and/or electron acceptor(s) and/or macromolecule(s) can be recognized by a person skilled in the art, regardless of the bond between the components, since, in principle, the redox-active centers (cofactors, prosthetic groups) and the affiliated matrix comprising macromolecule(s) (e.g. the apoprotein in the case of enzymes, as an example of a catalytically redox-active moiety) may also occur separately. The catalytically redox-active moiety may be for example any redox-active protein/enzyme from the group of oxidases or reductases; from this group of oxidases or reductases, proteins/enzymes modified by protein engineering or gene mutation; or an artificially produced moiety comprising one or more redox-active centers (electron donor or acceptor) or an artificially produced moiety comprising one or more redox-

	active centers (electron donor or acceptor) and one or more macromolecules binding these redox-active centers.
cofactor	Equivalent to a redox-active center (electron donor or acceptor) of the catalytically redox-active moiety.
prosthetic group	Equivalent to a redox-active center (electron donor or acceptor) of the catalytically redox-active moiety.
redox-active center of the catalytically redox-active moiety	The redox-active center of the catalytically redox-active moiety is characterized in that it acts as an electron donor or acceptor toward a substrate specific to the catalytically redox-active moiety. Moreover, if a catalytically redox-active moiety possesses multiple redox-active centers (electron donors and/or electron acceptors), a charge transfer may occur within the catalytically redox-active moiety: following the charge transfer between the substrate specific to the catalytically redox-active moiety and a first redox-active center, an additional charge transfer is possible between this first redox-active center and an additional redox-active center of the same catalytically redox-active moiety, this second redox-active center, in turn, being able to transfer charge to a third redox-active center, and so on. Thus, a successive charge transfer may occur within the catalytically redox-active moiety if the catalytically redox-active moiety includes multiple redox-active centers. In this case, the process of successive charge transfer is initiated by the presence of the substrate (with its property of spontaneously transferring a charge between the substrate and the catalytically redox-active moiety) specific to the catalytically redox-active moiety.
electron-donor molecule	Equivalent to an electron donor.
electron-acceptor molecule	Equivalent to an electron acceptor.
electron donor	In the context of the present invention, the term "electron donor" refers to a component of the catalytically redox-active moiety. An electron donor is a molecule that can transfer an electron to an electron acceptor, directly or under the influence of certain external conditions. For example, one such external condition is the oxidation or reduction of the electron donor or acceptor of the

catalytically redox-active moiety by an external oxidizing or reducing agent, so for example the transfer of an electron to the electron donor by a reducing agent, or the release of an electron by the electron acceptor to an oxidizing agent. These oxidizing or reducing agents may be external redox-active substances, i.e. they are not covalently joined with but in contact with the catalytically redox-active moiety, the nucleic acid oligomer, or the conductive surface, for example via the solution added to the modified conductive surface, the substrates specific to the catalytically redox-active moiety, in particular, being able to act as external oxidizing or reducing agents. In addition, an external oxidizing or reducing agent also may be covalently joined with the nucleic acid oligomer, the oxidizing or reducing agent being covalently attached to the nucleic acid oligomer at a site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the redox-active moiety, preferably at the oligonucleotide end opposite the modification with the catalytically redox-active moiety, near the conductive surface. In particular, also the conductive surface (electrode) may act as the external oxidizing or reducing agent. The ability to act as an electron donor or acceptor is relative, i.e. a molecule that acts as an electron donor toward another molecule, directly or under the influence of certain external conditions, may also act as an electron acceptor toward that molecule under differing experimental conditions, or toward a third molecule under the same or differing experimental conditions.

electron acceptor

In the context of the present invention, the term "electron acceptor" refers to a component of a catalytically redox-active moiety. An electron acceptor is a molecule that can take up an electron from an electron donor, directly or under the influence of certain external conditions. For example, one such external condition is the oxidation or reduction of the electron donor or acceptor of the catalytically redox-active moiety by an external oxidizing or reducing agent, so for example the transfer of an electron to the electron donor by a reducing agent, or the release of an electron by the electron acceptor to an oxidizing agent. These oxidizing or reducing

agents may be external redox-active substances, i.e. they are not covalently joined with but in contact with the catalytically redox-active moiety, the nucleic acid oligomer, or the conductive surface, for example via the solution added to the modified conductive surface, the substrates specific to the catalytically redox-active moiety, in particular, being able to act as external oxidizing or reducing agents. In addition, an external oxidizing or reducing agent can also be covalently joined with the nucleic acid oligomer, the oxidizing or reducing agent being covalently attached to the nucleic acid oligomer at a site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the catalytically redox-active moiety, preferably at the oligonucleotide end opposite the modification with the redox-active moiety, near the conductive surface. In particular, also the conductive surface (electrode) may act as the external oxidizing or reducing agent. The ability to act as an electron acceptor or donor is relative, i.e. a molecule that acts as an electron acceptor toward another molecule, directly or under the influence of certain external conditions, may also act as an electron donor toward that molecule under differing experimental conditions, or toward a third molecule under the same or differing experimental conditions.

oxidizing agent

A chemical compound (chemical substance) that oxidizes another chemical compound (chemical substance, electron donor, electron acceptor) by taking up electrons from this other chemical compound (chemical substance, electron donor, electron acceptor). An oxidizing agent behaves analogously to an electron acceptor, but is used in the context of the present invention to denote an external electron acceptor not directly belonging to the catalytically redox-active moiety. In this context, "not directly" means that the oxidizing agent is either a substrate specific to the catalytically redox-active moiety, or a free redox-active substance that is not bound to but in contact with the nucleic acid oligomer. In addition, the oxidizing agent may be covalently attached to the nucleic acid oligomer, but at a nucleic acid oligomer site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine

	bases away from the covalent attachment site of the catalytically redox-active moiety. In particular, the electrode also may represent the oxidizing agent.
reducing agent	A chemical compound (chemical substance) that, by giving up electrons to another chemical compound (chemical substance, electron donor, electron acceptor), reduces this other chemical compound (chemical substance, electron donor, electron acceptor). A reducing agent behaves analogously to an electron donor but is used in the context of the present invention to denote an external electron donor not directly belonging to the catalytically redox-active moiety. In this context, "not directly" means that the reducing agent is either a substrate specific to the catalytically redox-active moiety, or a free redox-active substance that is not bound to but in contact with the nucleic acid oligomer, or that the reducing agent is covalently attached to the nucleic acid oligomer, but at a nucleic acid oligomer site that is situated at least two covalently-joined nucleotides or at least two covalently-joined pyrimidine or purine bases away from the covalent attachment site of the redox-active moiety. In particular, the electrode also may represent the reducing agent.
redox-active	Redox-active refers to the property of a redox-active moiety of giving up electrons to a suitable oxidizing agent or taking up electrons from a suitable reducing agent under certain external conditions, or the property of a redox-active substance of giving up electrons to a suitable electron acceptor or taking up electrons from a suitable electron donor under certain external conditions.
analyte	Equivalent to a substrate.
substrate	A free oxidizing or reducing agent not covalently joined with but in contact with the catalytically redox-active moiety, the nucleic acid oligomer, or the conductive surface, for example via the solution applied to the modified conductive surface, the substrate being able to be for example a charged or uncharged molecule, any salt, an ion, or a redox-active protein or enzyme (oxido-reductase). The substrate is characterized in that it is recognized by the catalytically redox-active moiety due to the formation of specific

interactions between the substrate and the catalytically redox-active moiety and can reduce the donor (or oxidize the acceptor) of the catalytically redox-active moiety, the catalytic activity of the catalytically redox-active moiety accelerating (catalyzing) this redox reaction of the substrate to the product.

catalytic activity

The catalytic activity of the catalytically redox-active moiety has an accelerating effect on the specific reaction between the moiety and the affiliated substrate and thus allows a reaction course in which the catalytic activity of the moiety is imperceptible or nonexistent. This catalytic activity of the redox-active moiety is achieved by stabilizing the relevant transitional state, i.e. the highest-energy species, in the reaction course between the catalytically redox-active moiety and the affiliated substrate.

electrocatalytic activity

The electrocatalytic activity of the catalytically redox-active moiety is closely related to the catalytic activity of the moiety. The presence of the catalytically redox-active moiety and its integration in the course of the electrode reaction of the substrate to the product (the course of the entire electrochemical redox reaction between an electrode and the substrate, i.e. the electrode giving up electrons to the substrate or the substrate giving up electrons to the electrode, to the intermediate steps of the redox reaction between the substrate and the catalytically redox-active moiety and the redox reaction between the redox-active moiety and the electrode) accelerates the electrochemical conversion of the substrate at the electrode. The electrocatalytic activity of a catalytically redox-active moiety immobilized at an electrode reduces the activation energy of the electrode reaction of the substrate to the product (the energy of the highest-energy state for the course of the conversion of the substrate to the product at the electrode) and thus causes a shift in the electrode potential required for the electrode reaction of the substrate to the product, in the direction of the equilibrium potential for this electrode reaction. Decreasing the activation potential causes a reduction of the overpotential required for an electrode reaction, and thus an increase in the flow of electrons between the electrode and the substrate at a specific electrode potential that is suitable for the electrode reaction (this increase is generally referred to as "catalytic

current"). An important result of the electrocatalytic activity is thus that the electrochemical conversion of the substrate to the product can be carried out in the presence and with the participation of the catalytically redox-active moiety at an electrode potential at which, in the absence of the catalytically redox-active moiety, very little or no current flows.

specificity of the catalytically redox-active moiety

The catalytically redox-active moiety acts specifically both with a view to the substrate that interacts with the catalytically redox-active moiety and with a view to the reaction carried out with the relevant substrate. In the context of the present invention, redox reactions are the preferred reactions between the catalytically redox-active moiety and the substrate.

initiation process

Given appropriately chosen external conditions, the catalytically redox-active moiety exhibits its redox activity, in other words its property of for example giving up electrons to a suitable oxidizing agent or taking up electrons from a suitable reducing agent, only subsequent to an initiation process. Such an initiation process may be the addition of substrate with its property of transferring charge to the catalytically redox-active moiety: thus, the reductive property of a catalytically redox-active moiety becomes possible only through the transfer of the electron(s) from the substrate to the/an electron donor "D," either in the presence of an oxidizing agent that can oxidize D^- but not D, or because, subsequent to successive charge transfer within the catalytically redox-active moiety, the electron is transferred from D^- to an acceptor "A" (directly or via multiple electron transfer steps to intermediate electron acceptors) and an oxidizing agent is present that takes up electrons only from this reduced acceptor "A^-" of the catalytically redox-active moiety, but not from A. In particular, this oxidizing agent may also be an electrode, for example if the electrode is set to a potential at which D^- but not D (or A^- but not A) is oxidized. On the other hand, the oxidative property of a catalytically redox-active moiety becomes possible only through the transfer of the electron(s) from an electron donor "D" to the substrate, either in the presence of a reducing

	agent that can reduce D^+ but not D, or because, subsequent to successive charge transfer within the catalytically redox-active moiety, an electron is transferred from an acceptor "A" to the oxidized donor D^+ (directly or via multiple electron transfer steps from intermediate electron donors) and a reducing agent is present that gives up electrons only to this oxidized acceptor "A^+" of the catalytically redox-active moiety, but not to A. In particular, this reducing agent may also be an electrode, for example if the electrode is set to a potential at which D^+ but not D (or A^+ but not A) is reduced.
redox-active protein/enzyme	Usually consists of what is known as 'apoprotein,' the preferred macromolecule(s) of the present invention, and cofactors, the electron donor(s) and/or electron acceptor(s) within the meaning of the present invention. The redox activity of the redox-active protein/enzyme is triggered by a free redox-active substance (the specific substrate).
oxidase	A class of redox-active enzymes that catalyze the oxidation of the substrate specific to the relevant oxidase.
reductase	A class of redox-active enzymes that catalyze the reduction of the substrate specific to the relevant reductase.
oxido-reductases	Generic term for oxidases and reductases.
GOx	Glucose oxidase (β-D-glucose: oxygen 1-oxido-reductase, EC 1.1.3.4). An example of a redox-active protein/enzyme. The protein/enzyme is an enzyme comprising apoprotein and FAD as a cofactor, cf. Fig. 7 and Formula 1. The GOx is present as a homodimeric enzyme (Hecht et al., J. Mol. Biol. 229 (1993), pp. 153-172).
ADH	Alcohol dehydrogenase (EC 1.1.1.1). An example of a redox-active protein/enzyme. The protein/enzyme is an enzyme comprising apoprotein comprising three protein subunits and PQQ, heme, and a heme dimer as cofactors (Amayama et al., Methods Enzymol. 89 (1982) 450-457).
FDH	Fructose dehydrogenase (EC 1.1.99.11). An example of a redox-active protein/enzyme. The protein/enzyme is an enzyme comprising apoprotein and PQQ as (one of) the cofactor(s). The

	structure of this enzyme is unknown.
LDH	Lactate dehydrogenase (EC 1.1.1.27), an enzyme comprising apoprotein, FMN, and heme.
FAD	flavin adenine dinucleotide, cf. Formula 1
NAD^+	nicotinamide adenine dinucleotide, cf. Formula 2
PQQ	Pyrroloquinoline quinone; corresponds to 4,5-dihydro-4,5-dioxo-1H-pyrrolo-[2,3-f]-quinoline-2,7,9-tricarboxylic acid, cf. Formula 3 (R_1 = R_3 = R_5 = CO_2H; R_2 = R_4 = H) or a derivative thereof (Formula 3).
Heme	Iron-protoporphyrin IX, Formula 4 with $R_2 = R_5 = R_8 = R_{10}$ = H; $R_4 = R_6 = R_9 = R_{12} = CH_3$; $R_1 = R_3 = CH_2$-CH_2-CO_2^- ; $R_7 = R_9$ = $CH{=}CH_2$, or a derivative of iron-protoporphyrin (Formula 4).
N^6-(2-aminoethyl)-FAD	modified flavin adenine dinucleotide, cf. Formula 5
N^6-(2-aminoethyl)-NAD^+	modified nicotinamide adenine dinucleotide, cf. Formula 6
EDTA	ethylenediamine tetraacetate (sodium salt)
sulfo-NHS	N-hydroxysulfosuccinimide
EDC	(3-dimethylaminopropyl)-carbodiimide
HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
Tris	trishydroxymethylamino methane
alkyl	The term "alkyl" refers to a saturated hydrocarbon group that is straight-chain or branched (e.g. ethyl, 2,5-dimethylhexyl, or isopropyl, etc.). When "alkyl" is used to indicate a linker or spacer, the term refers to a group having two available valences for covalent linkage (e.g. $-CH_2CH_2-$, $-CH_2C(CH_3)_2CH_2CH_2C(CH_3)_2CH_2-$ or $-CH_2CH_2CH_2-$, etc.). Alkyl groups preferred as substituents or side chains R are those having a chain length of 1 - 30 (the longest continuous chain of atoms covalently bound to one another). Alkyl groups preferred as linkers or spacers are those having a chain length of 1 - 20, especially a chain length of 1 - 14, the chain length here representing the shortest continuous link between the structures joined via the linker or spacer, in other words between the two molecules or between a surface atom, surface molecule, or surface molecule

	group and another molecule.
alkenyl	Alkyl groups in which one or more of the C-C single bonds are replaced by C=C double bonds.
alkynyl	Alkyl or alkenyl groups in which one or more of the C-C single or C=C double bonds are replaced by C≡C triple bonds.
heteroalkyl	Alkyl groups in which one or more of the C-H bonds or C-C single bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
heteroalkenyl	Alkenyl groups in which one or more C-H bonds, C-C single or C=C double bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
heteroalkynyl	Alkynyl groups in which one or more of the C-H bonds, C-C single, C=C double or C≡C triple bonds are replaced by C-N, C=N, C-P, C=P, C-O, C=O, C-S, or C=S bonds.
linker	A molecular link between two molecules or between a surface atom, surface molecule, or surface molecule group and another molecule. Linkers can usually be purchased in the form of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, or heteroalkynyl chains, the chain being derivatized in two places with (identical or different) reactive groups. These groups form a covalent chemical bond in simple/known chemical reactions with the appropriate reaction partners. The reactive groups may also be photoactivatable, i.e. the reactive groups are activated only by light of a specific or any given wavelength. Preferred linkers are those having a chain length of 1 - 20, especially a chain length of 1 - 14, the chain length here representing the shortest continuous link between the structures to be joined, in other words between the two molecules or between a surface atom, surface molecule, or surface molecule group and another molecule.
spacer	A linker that is covalently attached via the reactive groups to one or both of the structures to be joined (see linker). Preferred spacers are those having a chain length of 1 - 20, especially a chain length of 1 - 14, the chain length representing the shortest continuous link between the structures to be joined.

	continuous link between the structures to be joined.
(n x HS-spacer)-oligo	A nucleic acid oligomer to which n thiol functions are each attached via a spacer, each spacer being able to have a different chain length (the shortest continuous link between the thiol function and the nucleic acid oligomer), especially any chain length between 1 and 14. These spacers, in turn, may be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been affixed thereto by modification, and "n" is any integer, especially a number between 1 and 20.
(n x R-S-S-spacer)-oligo	A nucleic acid oligomer to which n disulfide functions are each attached via a spacer, the disulfide function being saturated by any residue R. Each spacer for attaching the disulfide function to the nucleic acid oligomer may have a different chain length (shortest continuous link between the disulfide function and the nucleic acid oligomer), especially any chain length between 1 and 14. These spacers, in turn, may be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been affixed thereto by modification. The variable n is any integer, especially a number between 1 and 20.
oligo-spacer-S-S-spacer-oligo	Two identical or different nucleic acid oligomers that are joined with each other via a disulfide bridge, the disulfide bridge being attached to the nucleic acid oligomers via any two spacers, the two spacers being able to have differing chain lengths (the shortest continuous link between the disulfide bridge and the respective nucleic acid oligomer), especially any chain length between 1 and 14, and these spacers, in turn, being able to be bound to various reactive groups that are naturally present on the nucleic acid oligomer or that have been affixed thereto by modification.
mica	Muscovite lamina, a support material for the application of thin films.
Au-S-$(CH_2)_2$-ss-oligo-spacer-PQQ-FAD(GOx)	Gold film on mica having a covalently applied monolayer comprising derivatized 12-bp single-strand DNA oligonucleotide (sequence: TAGTCGGAAGCA **(SEQ ID NO.: 1)**). Here, the oligonucleotide's terminal phosphate group at the 3'-end is esterified with $(HO-(CH_2)_2-S)_2$ to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH, the S-S bond being homolytically cleaved and producing one Au-S-R bond each. The

	terminal thymine base at the 5'-end of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2, this residue, in turn, being joined via its free amino group with the carboxylic-acid group of the PQQ by amidation. To a further carboxylic-acid group of this PQQ is bound by amidation FAD that was previously modified in such a way that it has a reactive amino group at its disposal, for example by forming N^6-(2-aminoethyl)-FAD (Bückmann et al., 1991, European Patent 0.247.537.B1). Thereafter, the FAD is reconstituted with the apoprotein of the GOx such that a nucleic acid oligomer results that is covalently attached to the surface and, in addition – via PQQ as a covalently attached bridge molecule –, is covalently modified with the complete GOx moiety.
Au-S-(CH_2)$_2$-ds-oligo-spacer-PQQ-FAD(GOx)	*Au-S-(CH_2)$_2$-ss-oligo-spacer-PQQ-FAD(GOx)* hybridized with the oligonucleotide that is complementary to the ss-oligo (sequence: TAGTCGGAAGCA **(SEQ ID NO.: 1)**).
Au-S-(CH_2)$_2$-ss-oligo-spacer-PQQ-NAD^+-LDH	Gold film on mica having a covalently applied monolayer comprising derivatized 12-bp single-strand DNA oligonucleotide (sequence: TAGTCGGAAGCA **(SEQ ID NO.: 1)**). Here, the oligonucleotide's terminal phosphate group at the 3'-end is esterified with (HO-(CH_2)$_2$-S)$_2$ to form P-O-(CH_2)$_2$-S-S-(CH_2)$_2$-OH, the S-S bond being homolytically cleaved and producing one Au-S-R bond each. The terminal thymine base at the 5'-end of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2, this residue, in turn, being joined via its free amino group with the carboxylic-acid group of the PQQ by amidation. To a further carboxylic-acid group of this PQQ is bound by amidation NAD^+ that was previously modified in such a way that it has a reactive amino group at its disposal, for example by forming N^6-(2-aminoethyl)-NAD^+ (Bückmann et al., 1991, European Patent 0.247.537.B1). The complete LDH is associated at this terminal NAD^+.
Au-S-(CH_2)$_2$-ds-oligo-spacer-PQQ-NAD^+-*LDH*	*Au-S-(CH_2)$_2$-ss-oligo-spacer-PQQ*-NAD^+-*ADH* hybridized with the oligonucleotide that is complementary to the ss-oligo (sequence: TAGTCGGAAGCA **(SEQ ID NO.: 1)**).
E	The electrode potential on the working electrode.
E^{eq}	The equilibrium potential of an electrode reaction.

0	The "zero current" potential of an electrode reaction, the potential that does not supply total current (the sum of the oxidative and reductive current) for a specific electrode reaction.
h	The overpotential of an electrode reaction.
Electrode reaction	The redox reaction between a redox-active substance and an electrode (taking up electrons from the electrode by the redox-active substance or giving up electrons from the redox-active substance to the electrode).
E_{Ox}	The potential at maximum current of the oxidation of a reversible electrooxidation or electroreduction.
E_{Red}	The potential at maximum current of the reduction of a reversible electrooxidation or electroreduction.
I	current density (current per cm^2 of electrode surface)
cyclic voltammetry	Recording a current-voltage curve. Here, the potential of a stationary working electrode is changed linearly as a function of time, starting at a potential at which no electrooxidation or electroreduction occurs, up to a potential at which a species that is dissolved or adsorbed on the electrode is oxidized or reduced (i.e. a current flows). After running through the oxidation or reduction operation, which produces in the current-voltage curve an initially increasing current and, after reaching a maximum, a gradually decreasing current, the direction of the potential feed is reversed. The behavior of the products of electrooxidation or electroreduction is then recorded in a reverse run.
amperometry	Recording a current-time curve. Here, the potential of a stationary working electrode is set, for example by a potential jump, to a potential at which the electrooxidation or electroreduction of a dissolved or adsorbed species occurs, and the flowing current is recorded as a function of time.
potentiometry	Recording an electrode voltage course as a function of, for example, substrate consumption. Here, the potential of a stationary working electrode is set, for example, to the "zero current" potential E^0 of the substrate. When the substrate is consumed by the catalytically redox-active moiety (in the case of hybridization), the "zero current" potential E^0 changes in the direction of the equilibrium potential E^{eq}.

Thus, recording the potential as a function of time (~ substrate consumption) provides information on the hybridization state.

[074] Fig. 4 Shows a detailed schematic diagram of the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-FAD(GOx) of Figs. 3a-3c having gold as the surface material, mercaptoethanol as the spacer (-S-CH_2CH_2- spacer) between the electrode and the oligonucleotide, and -CH_2-CH=CH-CO-NH-CH_2-CH_2-NH-PQQ-NH-CH_2-CH_2- as the spacer between the cofactor FAD and the oligonucleotide, as well as a diagram of the sequence of the substrate-induced electron transfer steps. The apoprotein of the GOx is indicated only as a shell (solid line) (cf. Fig. 7). The 12-bp probe oligonucleotide of the exemplary sequence 5'-TAGTCGGAAGCA-3' **(SEQ ID NO.: 1)** in the hybridized state is shown in detail;

[076] Fig. 6 Shows a detailed schematic diagram of the surface hybrid Au-S$(CH_2)_2$-ds-oligo-spacer-PQQ-NAD^+-LDH of Fig. 3 having gold as the surface material, mercaptoethanol as the spacer (-S-CH_2CH_2- spacer) between the electrode and the oligonucleotide, and -CH_2-CH=CH-CO-NH-CH_2-CH_2-NH-PQQ-NH-CH_2-CH_2- as the spacer between the NAD^+ and the oligonucleotide to which ADH is associated, as well as a diagram of the sequence of the substrate-induced electron transfer steps. The 12-bp probe oligonucleotide of the exemplary sequence 5'-TAGTCGGAAGCA-3' **(SEQ ID NO.: 1)** in the hybridized state is shown in detail.

[090] For incubation, a doubly modified 12-bp single-strand oligonucleotide having the sequence 5'-TAGTCGGAAGCA-3' **(SEQ ID NO.: 1)** was used, which is esterified with $(HO-(CH_2)_2-S)_2$ at the phosphate group of the 3'-end to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH. At the 5'-end, the terminal thymine base of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2. Approximately 10^{-4} to 10^{-1} molar 2-hydroxy-mercaptoethanol (or another thiol or disulfide linker having a suitable chain length) was added to a $2x10^{-4}$ molar solution of this oligonucleotide in HEPES buffer (0.1 molar in water, pH 7.5 with 0.7 molar addition of TEATFB, see abbreviations) and the gold surface of a test site was completely wetted and incubated for 2-24 hours. During this reaction time, the disulfide

spacer P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH of the oligonucleotide is homolytically cleaved. In this process, the spacer forms a covalent Au-S bond with Au atoms of the surface, thus causing a 1:1 coadsorption of the ss-oligonucleotide and the cleaved 2-hydroxy-mercaptoethanol. The free 2-hydroxy-mercaptoethanol that is also present in the incubation solution is likewise coadsorbed by forming an Au-S bond (incubation step).

[096] For incubation, a doubly modified 12-bp single-strand oligonucleotide having the sequence 5'-TAGTCGGAAGCA-3' **(SEQ ID NO.: 1)** was used, which is esterified with (HO-$(CH_2)_2$-S$)_2$ at the phosphate group of the 3'-end to form P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH. At the 5'-end, the terminal thymine base of the oligonucleotide is modified at the C-5 carbon with -CH=CH-CO-NH-CH_2-CH_2-NH_2. Approximately 10^{-4} to 10^{-1} molar 2-hydroxy-mercaptoethanol (or another thiol or disulfide linker having a suitable chain length) was added to a 2×10^{-4} molar solution of this oligonucleotide in HEPES buffer (0.1 molar in water, pH 7.5 with 0.7 molar addition of TEATFB, see abbreviations) and the gold surface of a test site was completely wetted and incubated for 2-24 hours. During this reaction time, the disulfide spacer P-O-$(CH_2)_2$-S-S-$(CH_2)_2$-OH of the oligonucleotide is homolytically cleaved. In this process, the spacer forms a covalent Au-S bond with Au atoms of the surface, thus causing a 1:1 coadsorption of the ss-oligonucleotide and the cleaved 2-hydroxy-mercaptoethanol. The free 2-hydroxy-mercaptoethanol that is also present in the incubation solution is likewise coadsorbed by forming an Au-S bond (incubation step).